

Effects of nitrous oxide on the functional characteristics of cells in the extrastriate cortex of the cat*

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Summary. The directional preferences, receptive field areas, peristimulus-time (PST) histograms and spontaneous activities of 112 feline visual cortical (area 18) cells were studied before, during, and after the administration of nitrous oxide. These cellular characteristics were altered by nitrous oxide inhalation; some quite substantially. The data indicate that the functional characteristics of cortical visual cells, such as the receptive field and the directional preference, are variable; and, among other factors, depend also on the anaesthetic administered to the animal.

Key words: Nitrous oxide – Visual cortical cells – Cat

Introduction

Nitrous oxide (N_2O) was first recommended for surgical anaesthesia by Sir Humphrey Davy in 1799. Over the past thirty years, it has gained popularity as a convenient, non-explosive, easily-excreted anaesthetic; and it has been considered to be suitable for use in electrophysiological procedures in cats. However, Hammond (1971) felt that even an 80% concentration of N_2O did not provide sufficient anaesthesia in cats, and considered that supplemental small doses of Nembutal® were necessary. This contradicted the claim of Venes et al. (1971), that even 67% N_2O produces sufficient sedation and analgesia. Recently, Mandl et al. (1980) reported that, in the cat, N_2O modifies, or even abolishes, the responses of single neurons in the superior colliculus and striate cortex, as well as the responses of some fibres in the optic tract. They observed that the unit

activity elicited by flashing and moving stimuli was reduced, or completely obliterated; that the directional preference of the cells was modified; that the spontaneous discharge rate of the cells either decreased or dramatically increased; and that the unit's temporal spike distribution during stimulation was altered. About 28% of the visual cortical cells were affected by N_2O . In previous papers (Lahue et al. 1981; Reinis et al. 1983, 1984), it was found that the directional preference, receptive field area and other extrastriate cortical cell characteristics are considerably altered by vestibular stimulation. Since some of those experiments were done under N_2O anaesthesia, it was felt important that the magnitude of the change in the extrastriate cortical cell characteristics induced by nitrous oxide should be determined.

Material and methods

Twenty-two cats of both sexes were used in these experiments. One hundred and twelve visual cortical cells were studied.

The method for preparation of the animals has been described in detail in a previous paper (Reinis et al. 1984). In brief, a T-shaped Plexiglass mounting block was attached to the anterior portion of the skull of the cat in an operation performed two weeks before the first experimental session (anesthetics used: xylazine (Rompun®), 2 mg/kg; ketamine hydrochloride (Ketaset®), 30 mg/kg). Subsequently, the animal's head could be oriented in a standard position by clamping the block in a stereotaxic apparatus, thereby eliminating the need for the painful pressure points induced by eye, ear, and mouth bars. On the day of the experimental session, the animal was anaesthetized with a halothane-oxygen mixture; and an intratracheal cannula coated with Xylocaine® gel and intravenous cannula were introduced, together with contact lenses and artificial pupils which enabled sharp retinal images to be realized. The light stimulus (a light bar 0.2° by 13.3°, 60.6 cd/m² light intensity above the background) was back projected over a screen in front of the cat. The exposed portions of the skull were covered with sterile Gelfoam®, and the wound margins were infiltrated with a long-lasting local anaesthetic (0.25% Marcaine®). The animal was immobilized with pancuronium bromide (Pavulon®, 0.013 mg/kg), the halothane anaes-

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thetia was terminated and artificial ventilation was established. The stability of the position of the eye balls was periodically tested by the projection of the optic disk onto the screen in front of the animal. The unit activity was recorded with an epoxylite-coated tungsten microelectrode (2–3 M Ω impedance) in Brodmann's area 18 (Otsuka and Hassler 1962). The experimental animals were used repeatedly.

At the end of each recording session, which lasted for about five hours, the cat was injected with a small amount of Ketaset[®] in order to re-establish a degree of light anaesthesia. The bone defect in the skull was then closed with bone wax, the scalp incision was sutured, and the Pavulon[®] was disconnected. Artificial ventilation was continued until the animal recovered the use of its skeletal muscles; then, 600,000 units of Bicillin[®] (benzathine penicillin G) were injected as an antibiotic regimen.

During all experimental sessions, the animal's general condition was monitored with respect to its electroencephalogram (EEG), heart rate, core temperature, and carbon dioxide expired through the tracheal cannula. The state of alertness was determined regularly from the predominant EEG frequency. All precautions were taken to eliminate pain and distress during the periods when the animals were not anaesthetized with N₂O. The increasingly-friendly behaviour of the animals toward the experimenters between experimental sessions indicated that the precautions taken were successful. (The protocol for these experiments conforms to the recommendations of the *Guide to the Care and Use of Experimental Animals*, Volumes 1 and 2, published by the Canadian Council on Animal Care (CCAC). Moreover, all phases of these experiments were carefully analyzed and approved by the CCAC, following their repeated on-site inspection of the procedures used for animal treatment.)

At the end of the last experimental session, the animal was deeply anaesthetized with Pentothal[®] and perfused with physiological saline and 10% buffered formalin. The location of the electrode tracks in area 18 of the cortex was examined histologically.

The recorded signals were amplified with standard techniques, and digitized with an analog-digital converter (Tecmar Labmaster); the data were evaluated and handled with an IBM PC computer.

The recording programs were designed to detect spikes of several heights, sort them, and store the data in separate files. In this experiment, more interest was given to well-separated responses of the individual nerve cells than to the population responses. Therefore, only one or two cells were selected and recorded by the computer in any one session. Two cells were recorded and evaluated simultaneously only when it was possible to clearly distinguish two different shapes and amplitudes of action potentials on the screen of the oscilloscope, and when the computer counted them as two different entities. At the beginning of each experiment, an approximate area of the receptive field of the cell being studied was plotted, using a hand-held ophthalmoscope. Then, in one group of cells, the spontaneous activity of the cell (or cells) was recorded in one minute intervals for a total duration of 60 min. After this period, the anaesthetic (66% N₂O; 34% O₂; flow rate at 4.5 l/min) was administered through the respiratory pump for 30 min. The nitrous oxide was then replaced with pure oxygen for 10 min, and with air for another 60 min. Afterwards, the experiment was terminated and the animal allowed to recover. Twenty-five cells were recorded from in this way. Another group of 25 cells was recorded from for 160 min with the animals breathing only air. This latter group served as a control.

In 62 other cells, the experiments were conducted in the following ways:

(i) In 16 cells, the receptive fields were plotted by the computer before exposure to the N₂O; then again, starting 15 min

after the onset of N₂O administration, and finally, one hour after termination of N₂O.

(ii) In a group of 15 cells, the time course of the change in the receptive field areas following the onset of N₂O was studied. These areas were plotted immediately before N₂O administration, and again, at 5, 10 and 15 min after the onset of N₂O. Since these times were too short for computer-aided plotting, a hand-held ophthalmoscope was used. In all cases, the optimal length of the light bar that stimulated the cell was determined first. Then, the receptive field area was defined as a rectangle, the dimensions of which were determined by the optimal length of the light bar and the length of the path over which the light bar stimulated the cell. Therefore, the receptive field area was determined only approximately, but rather quickly. These data were then statistically compared by an analysis of variance.

(iii) In a third group of 16 cells, the peristimulus-time (PST) histograms were recorded by the IBM PC starting 15 min before the administration of N₂O, and again, starting 15 min after the onset of N₂O.

(iv) In the remaining 15 cells, the directional preferences were recorded before, and starting 15 min after the onset of N₂O.

The areas of the receptive fields were plotted in the following way: A rectangle, with a side of 15° of visual angle, was drawn around the centre of the receptive field of the cell as detected by a hand-held ophthalmoscope. The surface of the rectangle was then divided into 15 1-degree wide segments (bands). A light bar, 1-degree long, moving along each band was used as the stimulus. The speed of the movement of the light bar on the screen was 3°/s. The responses of the cells were recorded and data accumulated during twenty subsequent sweeps of the light bar. Then, the 1-degree stimulus was moved to the next band, and the responses of the cell were recorded again. The display of these data demonstrated the shape and size of the receptive field area of the cell, including its excitatory and inhibitory regions. Each display is divided into 15 horizontal bands, each 1° of visual angle wide by 24° of visual angle long. The short vertical lines indicate the number of spikes/bin after 20 sweeps of the 1-degree-long light bar. The width of the bin is 0.06° of visual angle.

The PST histograms were recorded with the light bar moving across the screen in front of the animal at the optimal angle at which the cell responded with the highest number of spikes. The speed of the light bar in this experiment was 6°/s, and data from 100 sweeps were accumulated. The width of individual bins for data storage was 10 ms, and each record consisted of 800 bins for 8 s of data.

The directional preference was studied by moving the light bar across the receptive field of the cell at a speed of 12°/s at 24 different angles. The angular distance between the line directions was, therefore, 15°. The light bar was oriented at 90° to its direction of movement. Cell responses to ten sweeps were averaged. Immediately before the administration of N₂O, the directional preference was tested at least twice, in order to ensure that the cell's responses were invariant over time.

Results

All receptive fields were situated within 10° of the visual angle from the projection onto the screen of the optic axis of the eye that was being studied. Histological examination indicated that all tracks of the recording electrodes were located within area 18 of the cortex.

The results from recording the spontaneous activity of the control group are summarized in Fig. 1. As

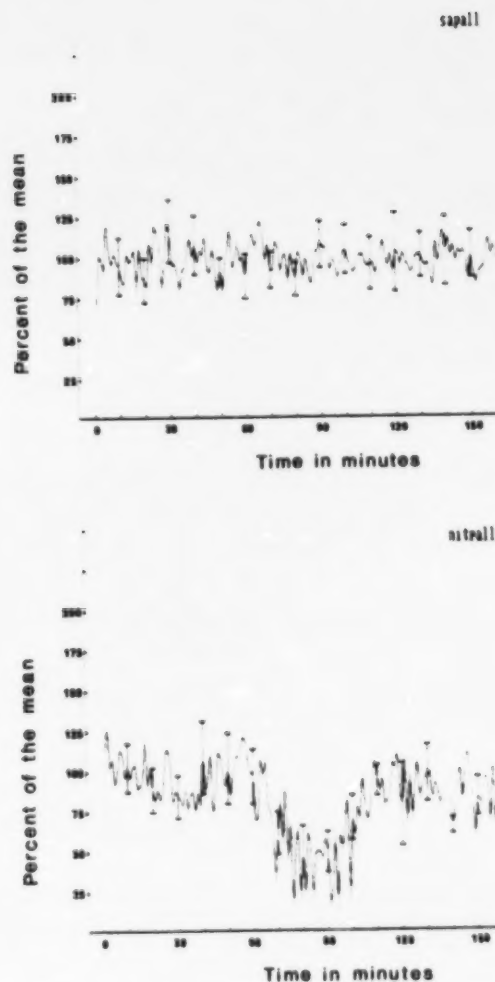


Fig. 1. Average values of the spontaneous firing rate of visual cortical cells for 160 min of experimentation. Upper curve: control group of 25 cells; lower curve: 25 cells exposed to nitrous oxide (between 60 and 90 min) followed by O_2 administration (between 90 and 100 min)

indicated, the average spontaneous activity of the 25 neurons did not change substantially during 160 min of recording. These average values, however, do not show the gradual increases and decreases in the spontaneous activities which were occasionally observed in the individual cells.

The administration of the N_2O caused a rapid decrease in the spontaneous activity in most cells that were studied. Of these 25 cells, a substantial decrease, or the disappearance of the spontaneous activity, was observed in 19 cells. In five cells, the spontaneous activity fluctuated, but did not decrease substantially; and, in one cell, the spontaneous firing

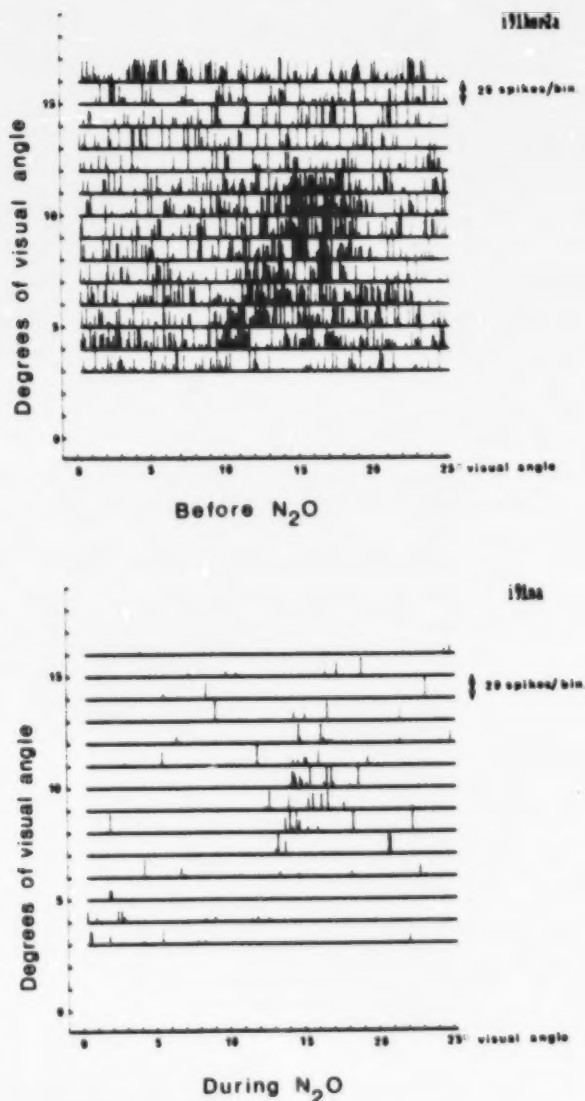


Fig. 2. Displays illustrating changes in the receptive field area in one cell before (upper figure) and during (lower figure) exposure to N_2O . Each display is divided into 15 horizontal bands, each 1° of visual angle wide by 24° of visual angle long. The short vertical lines indicate the number of spikes/bin after 20 sweeps of the 1-degree-long light bar. The width of the bin is 0.06° of visual angle. The distance between horizontal lines has a constant value of 20 spikes/bin. Divisions on the horizontal and vertical axes: degrees of visual angle

rate increased. Thus, the spontaneous activity of all observed cells was altered by nitrous oxide. The average curve of these cells is also presented in Fig. 1. In 23 cells, the spontaneous activity returned to pre-exposure levels. In two cells, the spontaneous

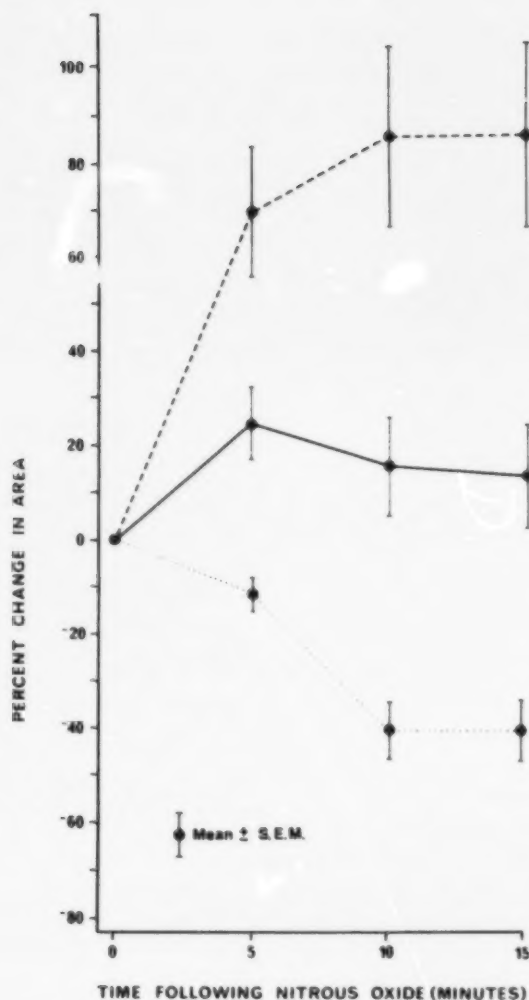


Fig. 3. An illustration of the percentage change in receptive field area following N_2O administration at time equal to 0 min. Shown are the mean $\pm$ standard error of the mean (S.E.M.) up to 15 min after N_2O administration for: (a) all 15 cells (—); (b) 6 cells in which the receptive field area increased (---); and (c) 7 cells in which the receptive field area decreased (.....). In 2 cells, there were no changes throughout the period of observation

activity temporarily increased above the pre-exposure level following the discontinuation of N_2O .

The receptive field areas of 15 observed cells in which N_2O was administered also changed; in some of them, substantially. In 3 cells, there was no observable change in the receptive field; whereas, in 12 others (80% of the observed cells), there was either some increase, or some decrease, or even a disappearance of the receptive field. Examples of these changes are illustrated in Fig. 2. In another

experiment, two neighbouring cells responded to N_2O administration, in one case, by an expansion of the receptive field, and, in the other, by the disappearance of the receptive field and a general decrease in the firing rate.

A study of the time course of the changes in the receptive field area showed that within 5 min after starting N_2O administration, the optimal length of the stimulating line changed, as did the path on which the line was able to excite the cell. In six of the observed cells, the receptive field area (which is a function of these two variables) expanded; in seven, it decreased; and, in two of the cells, there was no change. All together, changes in the receptive field areas were observed in 87% of cells. These data are presented in Fig. 3. After about 15 min exposure to N_2O , the receptive field areas of the cells stabilized at a new, altered size, after which there were no further changes.

In those cells in which the area shrank, the maximum decrease was by an average of 43.3%. In one case, the area completely disappeared and reappeared after N_2O was discontinued. In the 6 cells in which the receptive field area increased, it changed by an average of 84.1% at the maximum.

The ratio of the "width" to the "length" of the receptive fields was also calculated for these cells. In six cells, the ratios did not change substantially, even though the field increased or decreased in size. Four fields became slightly elongated, while five other fields were proportionately shorter compared to the pre-exposure dimensions.

The PST histogram was used to demonstrate the preferred times of firing of a cell to a series of stimulus presentations. Figure 4 illustrates that the effects of N_2O can be quite variable. For example, in Cell 140-1 the preferred time of firing is maintained during exposure, although at a somewhat reduced level, and with greater variability in the firing than before. In Cell 141-1, on the other hand, the N_2O substantially reduced the overall level of activity, and eliminated the single preferred firing time. In Cell 150-4, the preferred times of firing were reduced, although the frequency of spikes in the individual time bins was not substantially reduced. Results such as these indicate that the effects of N_2O on the visual cortex are quite variable.

The directional preferences of the observed cells were also altered by N_2O inhalation. It was noted in the set of cells that were studied (which, perhaps, resembles the complex cells of Hubel and Wiesel (1978)), that the directional preference is usually a rather complicated function, and some cells have a rather wide range of angles to which they will respond. Nitrous oxide did change the sensitivity of

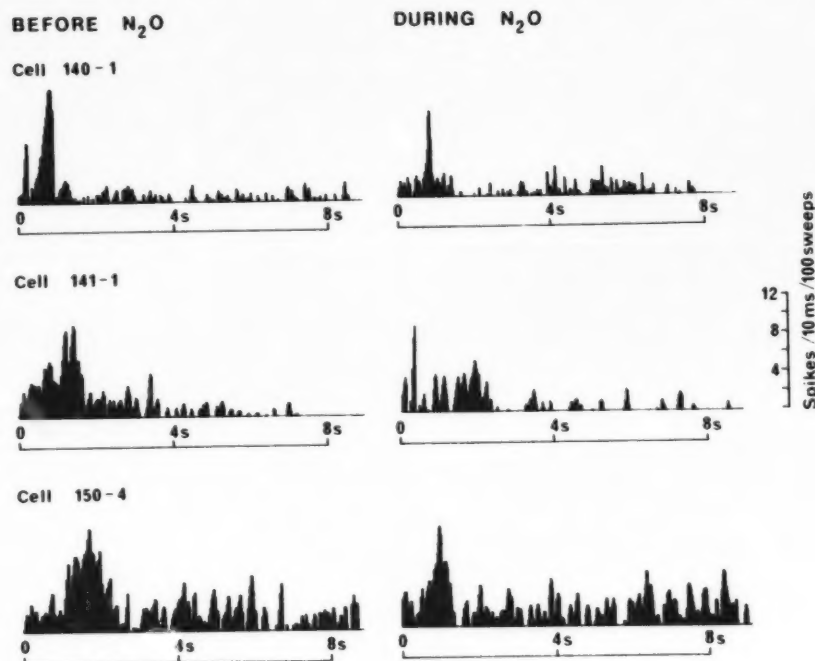


Fig. 4. Three examples of the changes in the PST histograms of complex visual cortical cells after inhalation of N_2O (see text for details). Units of time on abscissae are in seconds (s)

the cells to various angles of the light bar. Examples of such changes are shown in Fig. 5.

Initially, Cell 147-2 (Fig. 5A) responded exclusively to light bar presentations organized within a somewhat narrow range of specificity. Following the N_2O treatment, the directional specificity of this cell expanded; its responses became more diffuse, with no discernible preferred angles of light bar movement. The opposite effect occurred in Cell 148-2 (Fig. 5B), a cell which originally lacked a well-defined directional specificity. During N_2O administration, the specificity was reduced to only three angles of light bar movement which were capable of eliciting a response. Cell 150-4 (Fig. 5C) preferred a narrow range of specificity, both before and after the N_2O treatment; however, the pattern of specificity widened after N_2O exposure.

Discussion

In the main, the data recorded from area 18 of the cerebral cortex of the cat resemble the data of Mandl et al. (1980) obtained from the feline unit activity recorded in the superior colliculus and in area 17. However, the functional changes observed in area 18 affect most of the cells. Since Brodmann's area 18 is probably involved in a more complex analysis of the visual image than area 17, it may be conjectured that

the more complex the function of the cortical area, the more it is influenced by N_2O . Since, in each single cell, only one functional variable was examined at any one time, it was not possible to search for a covariance of the various nitrous-oxide effects. Also, the correlation between EEG changes and changes of functional characteristics of single cells was not examined systematically.

Otherwise, these data show that the use of N_2O as the anaesthetic in any study of cortical unit activity must be done with great caution, and that the data obtained from N_2O -anaesthetized animals must be compared with data from non-anaesthetized animals. A search for a more suitable anaesthetic and/or for new ways of pacifying animals during these types of experiments should be initiated.

Hammond (1978) has already expressed a suspicion that N_2O is not a suitable anaesthetic for experimentation with cats. The study described herein did not directly address this controversial statement. However, it has shown that all studies of unit activity in the brain using N_2O anaesthetic should be evaluated with caution. This is true also for the data published by the investigators in 1983 (Reinis et al. 1983), when N_2O was administered to immobilized cats. For this reason, data published in 1984 (Reinis et al. 1984), when N_2O was not used, reflect more closely the functional characteristics of the cortical visual cells.

Before N₂O

Cell 147-2

Recording Date 28 Sept '81

After N₂O



Cell 148-2

Recording Date 5 Oct '81



Cell 150-4

Recording Date 12 Nov '81

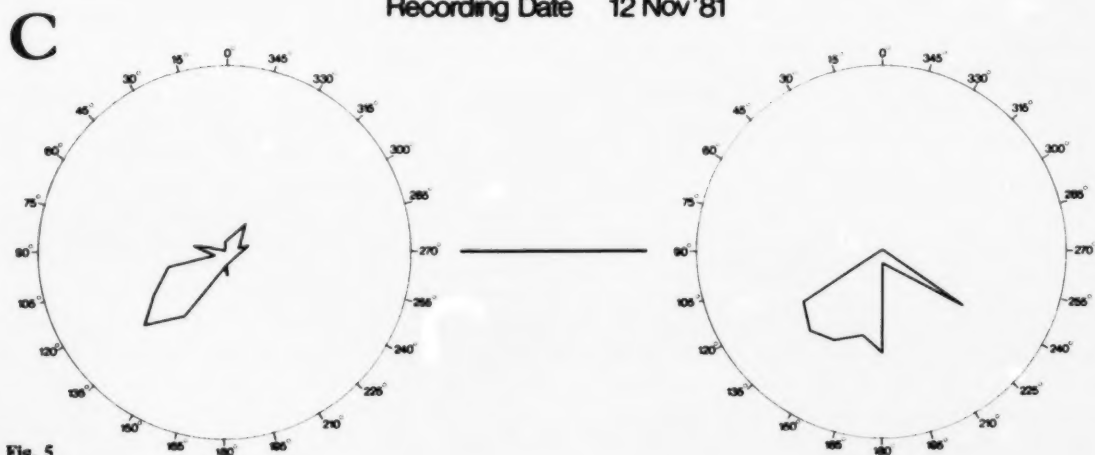


Fig. 5

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Fig. 5. Examples of the changes in the directional preference of complex visual cortical cells caused by inhalation of N_2O . Shown are the average numbers of impulses that were evoked above the spontaneous level, before and after the administration of N_2O , to 10 sweeps of a light bar moving at $12^\circ/s$ across the receptive field of three area-18 cells. The angles of the polar plots represent the 24 different sweeps of the light bar. The bars between each set of polar plots represent radial extents for those plots having magnitudes of 100 impulses/s. Further details are given in the text